Results: Pellets cultured in chondrogenic medium supplemented with TGF-β1 had a greater pellet area than those cultured in chondrogenic medium only ($p < 0.001$). The addition of TGF-β1 to the culture medium led to positive safranin-O staining and higher Bern scores compared to pellets cultured without TGF-β1 ($p < 0.001$). TβR-II intensities were greater in pellets stimulated with TGF-β1 than in unstimulated pellets ($p = 0.043$). Overall, positive correlations were observed between TβR-II total intensity and measured pellet area ($p = 0.033$), as well as between TβR-II intensity and BERN score ($p = 0.037$).
Conclusions: The results from this study indicate that equine MSC are responsive to TGF-β1 and can undergo chondrogenic differentiation. This was observed by the increase in pellet area and higher Bern scores in the presence of TGF-β1. In addition to these commonly reported measures, this study also evaluated the intensity of TβR-II following *in vitro* chondrogenic differentiation and showed that it increased in the presence of TGF-β1. This increase suggests that TβR-II may be an indicator of cellular responsiveness to TGF-β1. The positive correlation observed between TβR-II levels and BERN scores indicates that TβR-II expression is important in chondrogenesis and may be a novel predictor of MSC chondrogenic potential. Using TβR-II as an indicator of chondrogenic potential thus warrants further investigation as it may lead to improved cell-based therapies for articular cartilage repair, which may delay or prevent the development of OA after injury.

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IS THE SECOND MICROFRACTURE STILL USEFUL FOR REPAIR OF ARTICULAR CARTILAGE DEFECT?

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Purpose: Microfracture (MFx) is considered as the first-line treatment for full thickness cartilage lesion because of its minimal invasiveness, technical easiness and high cost-effectiveness. Owing to its advantages, it can be postulated that MFx can be repeated in spite of time dependant poor long-term follow-up result. The purpose of this study was to evaluate whether the second MFx could effectively repair the cartilage defect which was generated at the spot with previously MF being bone.

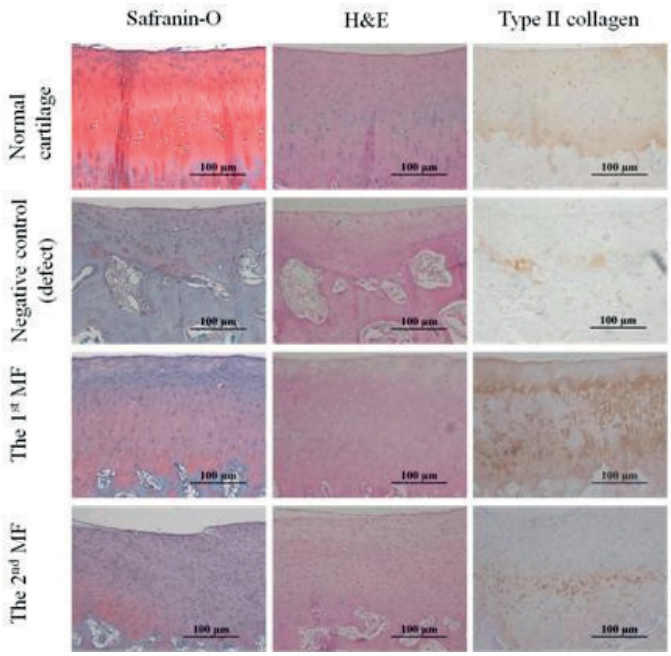

Fig. 1. Histologic evaluation of the repaired tissue 8 weeks after MFx. (A,E,I) normal, (B,F,J) control, (C,G,K) first MF, (D,H,L) second MF group.

Methods: Thirty-six New Zealand white rabbits were divided into 3 groups: (1) untreated full-thickness chondral defect, (2) treated with MFx and (3) the second MFx. The number of mesenchymal stem cells (MSCs) derived from bone marrow was evaluated by colony forming unit (CFU) assay. The repaired cartilage was evaluated 8 weeks after the first and second MFx. Cartilage evaluation was done by histology and biochemical assay and subchondral bone was analyzed by micro-CT.
Results: There was no significant difference in the colony formation between the first MFx and second MFx. The repaired cartilage after the second MFx was comparable with that after the first MFx in terms of histologic score and biochemical results even with slightly lower density of GAG and type II collagen. Subchondral bone remained severely damaged in both the first and second MFx.
Conclusions: The second MFx showed comparable results to the first MFx in spite of tendency to form lower quality of repaired cartilage in histologic study.

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CHONDROGENESIS DIFFERENTIATION OF MESENCHYMAL STEM CELLS FROM HUMAN AMNIOTIC FLUID CULTURED WITH TGF BETA 3

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Purpose: Recently, adult mesenchymal stem cells (MSCs) have been focused as an alternative source of cells for cartilage repair. It is accepted that amniotic fluid is a new source of MSCs, and has characteristics similar to embryonic stem cells which makes them a potential source for cell differentiation. Cellular condensation is a required step in the initiation of mesenchimal chondrogenesis. The aim of this study was to differentiate cells from amniotic fluid into chondrocytes in high density micromass culture to evaluate the expression of type II collagen.
Methods: Amniotic fluid from 44 pregnat women was harvested in the second semester. The samples were set in flasks and cultured. Adherent cells were selected and expanded until 4th passage to obtain the appropriate number of cells. The cells were analysed by flow cytometry and after this process, they were platted in high density micromass culture system, remaining under this condition by 3 weeks in Dulbecco's Modified Eagle Medium (DMEM) with high glucose and transforming growth factor-beta-3(TGF-beta 3)in a final concentration of 10 ng/ml in micromass culture. After 21 days, cell differentiation was verified by western blotting analysis of the secreted collagen II protein in the culture medium.
Results: Through flow cytometry, expanded cells showed typical cell surface antigens found in mesenchymal stem cells, such as positivity for CD90, and negativity for antigens found in haematopoetic lineage. After 21 days in high density micromass culture with TGF beta-3 containing medium, the expression of type II collagen was observed and confirmed by western blotting. fluid cells into chondrocytes.
Conclusions: The authors showed amniotic fluid MSCs can be differentiated in articular chondrocytes under the TGF-beta-3 stimuli, therefore can be used as a reliable source of MSCs.

Cell Signaling

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COMP BINDS TRANSFORMING GROWTH FACTOR BETA FAMILY LIGANDS TO ENHANCE THEIR ACTIVITY

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Purpose: Cartilage oligomeric matrix protein (COMP) is an important protein essential for the formation and maintenance of the structural integrity of cartilage matrix. COMP is a homo-pentamer in which each monomer comprises an N-terminal coiled-coil domain, epidermal growth factor (EGF) – like repeats, thrombospondin (TSP)-3 like repeats and a thrombospondin C-terminal domain. Owing to its repeated modular structure, COMP can assemble various extracellular matrix components like collagens and proteoglycans. We hypothesized that if COMP were to bind to growth factors, it could affect growth factor activity by controlling how and when the growth factors are presented to the cell surface. The aim of this study was to examine the mechanism and effect of binding

interactions between COMP with chondrogenic growth factors like TGFβ1 and bone morphogenetic proteins (BMPs), and whether this affects the cellular response to the growth factors.

Methods:

i. Solid phase ELISA binding assays determined the binding of COMP to TGFβ and BMP ligands using soluble COMP and immobilized TGFβ1, BMP-2, BMP-4 and BMP-7, and vice versa. Binding assays were also conducted for TGFβ1 and deletion constructs of COMP.

ii. ELISA was carried out to see the effect of divalent cations on COMP-TGFβ1 binding. A constant amount of COMP was allowed to bind with immobilized TGFβ1 in the presence of different concentrations of Ca, Mg, Mn or EDTA.

iii. TGFβ1 was labeled with gold thiocyanate, bound to COMP or an N-terminally truncated COMP construct in the presence or absence of manganese, and visualized by negative-stained electron microscopy.

iv. Luciferase assays were performed with a mink lung cell line stably transfected with a TGFβ-responsive promoter, and a C2C12 cell line stably transfected with a BMP-responsive promoter, driving the expression of luciferase. We measured: a) TGFβ1 dependent transcriptional activation by the COMP-TGFβ1 complex, and b) BMP-dependent transcriptional activation by the COMP-BMP7 complex.

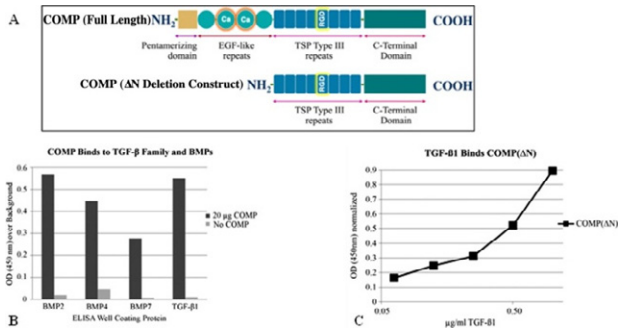

Fig. 1. (A) Schematic representation of COMP and one deletion construct. (B) Soluble COMP binds to the TGF-β superfamily proteins. (C) Deletion construct of COMP binds to TGF-β1 in a dose-dependent manner.

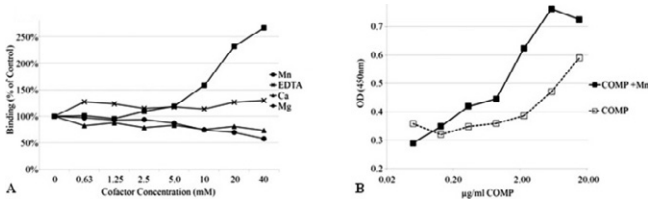

Fig. 2. (A) Increasing concentrations of manganese enhance the binding of COMP to TGF, while similar concentrations of Ca, Mg and EDTA do not affect the interaction. (B) Dose-dependent increase in the amount of COMP bound to TGF in the presence of Mn.

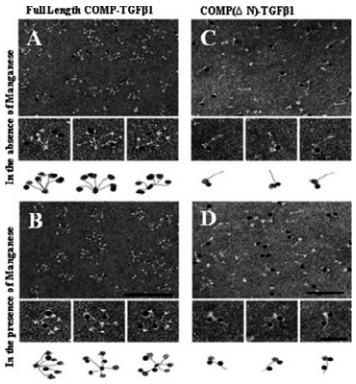

Fig. 3. Electron micrographs of TGF-β1 bound to : (A) COMP in the absence of manganese; (B) COMP in the presence of manganese; (C) deletion construct of COMP, COMP(ΔN), in the absence of manganese; (D) COM)(ΔN) in the presence of manganese. In B and D, an additional binding site is observed.

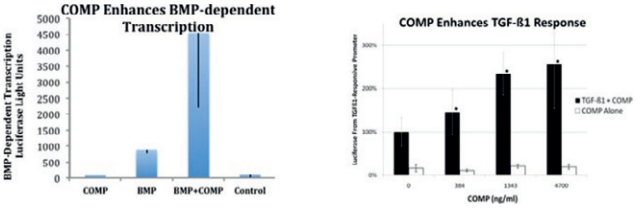

Fig. 4. Activation of BMP- and TGF-β1-dependent transcription. (A) COMP–BMP 7 binding enhances transcriptional activation of luciferase reporter in BMP responsive cells. (B) TGF-β1-dependent transcription is enhanced in a dose-dependent manner by TGF-β1 binding to increasing amounts of COMP. COMP alone has no effect on BMP-- or TGF-β1-dependent transcription.

Results:

i. Solid phase ELISA binding assays revealed that COMP bound to TGFβ1, BMP-2, BMP-4 and BMP-7 and half-maximal binding occurs with 1.4 μg of COMP and 1 μg of TGFβ1. The different deletion constructs of COMP also bound to TGFβ1 in a dose dependant manner (Figure 1).

ii. Addition of Manganese caused a dose-dependent increase in the amount of COMP binding to TGFβ1 (Figure 2).

iii. Electron micrographs show that TGFβ1 binds to the C-terminal of COMP and its deletion constructs. An additional TGFβ1 binding site is observed in the presence of manganese (Figure 3).

iv. Luciferase assays showed that while COMP alone had no effect on transcriptional activation, it was enhanced in a dose-dependent manner when increasing amounts of COMP were bound to fixed amounts of TGFβ1 or BMP7 (Figure 4).

Conclusions: Our studies show that there is a direct binding between COMP and TGFβ ligands and this binding can be enhanced in the presence of a divalent cation, manganese. TGFβ ligands seem to bind to the C-terminal domain of COMP while addition of manganese opens up yet another binding site, as also seen in the COMP deletion construct. COMP bound to these ligands causes transcriptional activation by increasing the cellular response to TGFβ1 and BMP.

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ELEVATED ENDOGENOUS SCLEROSTIN LEVELS IN OSTEOARTHRITIS SUBCHONDRAL OSTEOBLASTS CONTRIBUTE TO REDUCED WNT/B-CATENIN SIGNALING IN THESE CELLS

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Purpose: Clinical and *in vitro* studies suggest that subchondral bone sclerosis due to abnormal osteoblast (Ob) function, is involved in the progression and/or onset of osteoarthritis (OA). Moreover, human OA subchondral Ob show a phenotype of very differentiated cells, however they fail to mineralize normally *in vitro* as *in vivo*. Wnt signaling plays a key role in osteogenesis by promoting the differentiation and mineralization of Ob mainly via the canonical Wnt/β-catenin (cWnt) signaling pathway. Sclerostin (SOST) has been shown to alter cWnt signaling, however the regulation of SOST in OA Ob remains unknown. Here we investigated the role of SOST in OA Ob.

Methods: We prepared primary human subchondral Ob using the sclerotic medial portion of the tibial plateaus of OA patients undergoing knee arthroplasty, or from tibial plateaus of normal individuals at autopsy. SOST expression and production was evaluated by qRT-PCR and WB analysis. The regulation of SOST expression was determined in response to transforming growth factor-β1 (TGF-β1) and as a function of the growth of OA Ob. SOST inhibition was performed using siRNA techniques. cWnt signaling was evaluated by measuring target gene expression using the TOPflash Tcf/lef luciferase reporter assay and intracellular β-catenin levels by WB. Mineralization was evaluated by Alizarin red staining. TGF-β1 levels were determined by ELISA.

Results: SOST expression was high in OA Ob compared to normal. Western blot analysis also indicated a similar distribution for OA Ob compared to normal Ob. SOST expression and production increased in post-confluent OA Ob and normal Ob, however its increase was more pronounced in OA Ob and levels remained always higher in OA Ob. TGF-β1 expression was high in OA Ob and stimulated SOST expression and production in Ob. cWnt signaling was reduced in OA compared to normal Ob. Inhibiting SOST expression by siRNA increased cWnt signaling